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The Effects of Oral Controlled Exposure on Colostrum Characteristics in Swine

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by

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Dedication

I dedicate this dissertation to my cherished parents Jair and Sandra, friends and Alissa. To my parents and sister who have supported me through long conversations over the phone and by the couple of visits they made to see me (from 6000 miles away). To my friends in Ames who were a big part of my life and made my life a lot more enjoyable here. Lastly to Alissa who was by my side in bad and good moments always respecting me and helping me out along the way.

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ABSTRACT

The first chapter is composed of a literature review including topics such as basic porcine immunology, placenta physiology, colostrum composition and immunological importance and lastly a general review about *Clostridium perfringens*-associated disease in piglets. The objective of this chapter was to build a solid scientific background to support the two following research topics. The second chapter is composed of the oral control exposure trial and the third chapter reviews the *Clostridium perfringens* transmission in pigs.

Oral exposure with herd-derived animal materials has been widely used in swine production over the years to stimulate herd immunity as a method to control and prevent diseases. The process consists of an intentional exposure of breeding age females to feces, tissues, or environmental materials, presumably containing potential pathogens, with the objective of increasing immunity thus decreasing clinical disease. In 1953, Bay concluded that sows fed infective intestinal material with a coronavirus (Transmissible Gastro Enteritis virus) at various intervals before farrowing were capable of transferring passive immunity to their litters. Years later Kohler`s (1974) study demonstrated that feeding back enteropathogenic E. coli to sows during the last month of gestation increased the anti-diarrheagenic value of colostrum and milk.

Accurate diagnosis and control of neonatal diarrhea is complicated by interactions of infectious agents and risk factors. Intentional feedback of farrowing house-derived materials (usually feces) given to pregnant females' weeks before farrowing has the objective of bolstering both the pre-farrow immunity and post-farrow passive transfer of protective antibodies to the offspring. This concept is particularly adaptable to swine due in part to species specific attributes and modern confinement housing systems

Despite wide use of controlled oral exposure over in swine the past 40 years, there is a general lack of scientific information available supporting this intervention other than *E. coli* and Transmissible Gastroenteritis (TGE) virus control. This lack of scientific data and mixed results in the field when attempting to control other pathogens has generated much controversy among practicing veterinarians. The objectives of this study were to better

characterize the oral controlled exposure (OCE) methodology, to determine the optimal timing of OCE and to evaluate whether or not biological parameters such as total colostral immunoglobulin G, white cells and some specific antibodies are suitable to monitor the efficacy of the OCE process. The shedding of microorganisms was also assessed to identify if this procedure has any influence on the shedding pattern.

The third chapter is a literature review about *Clostridium perfringens* (*Cp*) disease in piglets. The review focuses on *Cp* type A and type C which are associated with disease in neonatal piglets. The first part of the review describes the nature of the disease and how the different types are categorized. The second part describes the clinical signs and findings associated with the two different pathogens. The primary focus of the review is transmission of the agent and possible methods of control. Topics such as first colonization, gut microflora succession and shedding are discussed due to their relevance. The last part of the chapter discusses the different possibilities of control including several different methodologies such as vaccination, oral controlled exposure, the use of antibiotics, probiotics and prebiotics, and lastly an emphasis on cleaning and disinfection.

CHAPTER 1. DISSERTATION ORGANIZATION

This thesis begins with a review of literature about specific topics which were considered essential in understanding the biology as well as providing a background of specific information used in the development of the trial and finally how this information was used to meet the study objectives. The literature review contains information regarding the background, description and definition of the oral controlled exposure, basic swine immunology with an emphasis on the physiology of the swine placenta, role of colostrum and ecology of *Clostridium perfringens* associated enteric disease in piglets.

Subsequent to the literature review is one manuscript prepared for publication in a scientific journal. In the experiment conducted, gilts received either treatment or no treatment. The treatment consisted of repeated oral controlled exposure during the study period. The goal of this study was to evaluate the efficacy of the oral controlled exposure on measures of immunity, transfer of passive immunity through colostrum and shedding of Clostridium in sow feces at farrowing. The treatment was applied in different timelines with the objective of verifying if time and duration of treatment would affect potential outcomes. The third part of this thesis consists of a manuscript prepared for a publication at *Journal of Swine Health and Production*. The manuscript is a literature review of *Clostridium perfringens* in pigs with special emphasis on transmission focusing on the development of better strategies to control the disease.

The thesis finishes with a general conclusion chapter and discussion of the results of the experiment and its implications. This also includes a discussion on the likely positive and negative effects when using of this technique as a method to control and prevent neonatal disease in swine production.

CHAPTER 2. THE HISTORY OF ORAL CONTROLLED EXPOSURE IN SWINE, APPLICATIONS IN VETERINARY MEDICINE AND BASIC ASPECTS OF PORCINE IMMUNOLOGY

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Introduction

In the 12th century, smallpox spread widely in Europe and Asia. Many people were afflicted and died from the disease. In China, it was noticed that some of the individuals who recovered from the disease were resistant to subsequent infection. Based on that observation, children were purposely and primitively infected with smallpox so that those who survived were protected against the disease in later life (Tizard, 2009). This is the first report outlining intentional exposure in order to develop immunity (immunization). In 1879, the French scientist Louis Pasteur, while investigating fowl cholera caused by *Pasteurella multocida* (PM), made a very similar and important discovery. In one of his experiments, Pasteur had a culture of PM which was accidentally allowed to become old in the laboratory. When the aged culture was used to infect chickens, a surprising thing happened: the chickens remained healthy and free of any symptoms. Pasteur then decided to use those same chickens again. He inoculated the birds a second time using a fresh culture of PM fully expecting to reproduce disease. To his surprise, the inoculated chickens did not die or even develop the symptoms of fowl cholera. In a brilliant conclusion, Pasteur recognized that the chickens exposed to an aged (attenuated) culture of an organism do not develop the disease and are subsequently resistant to disease (Tizard, 2009). This observation introduced the concept of immunity, and that some type of exposure response could protect the animal against a subsequent infection by a virulent strain of the same microorganism. Through his conclusions, Pasteur established the general principle of vaccination (immunization). Pasteur`s discoveries were very important to the development of vaccines and other techniques against many infectious agents (Tizard, 2009).

Scientists have known for over 100 years (Tizard, 2009) that animals can develop immunity

against certain diseases if properly exposed to the killed infectious agent, to low doses of an agent, or to a modified live but avirulent form of the agent. In addition, these concepts have contributed to the development of other immune modulation techniques; oral controlled exposure with feces is one of them. Results derived from orally administrated pathogen studies were described in a review in 1927 (Besredka, 1927) and have been the subject of several additional studies over the years (Keren, 1992; Rimoldi, 2005; Weiner, 1997). In 1947 researchers reported an induction of active immunity against an enteric disease of guinea pigs caused by *Vibrio cholera* after they were orally inoculated with the live bacteria (Burrows, 1947). Similar responses were observed in mice orally administrated a live culture of *Salmonella enteritidis* (Collins, 1972) and monkeys inoculated with resistant strains of *Escherichia coli* (Felsenfeld, 1972). All of these classic studies investigated the immunological protection of an individual animal after oral administration of a pathogen. None of the studies explored the possibility of protection of offspring via the passive immune mechanism involved in maternal transfer.

In the 1960's, veterinary studies began to explore different immunological approaches and the ramifications when immunization was applied to animal populations. Included in these early reports were the benefits of colostrum from vaccinated sows on their offspring. The hypothesis was tested experimentally by infecting gnobiotic piglets with or without supplemental colostrum from vaccinated sows (Svendsen and Wilson, 1971). In 1971, studies demonstrated that colostrum and milk whey fed to piglets after farrowing increased the survival rate in gnobiotic pigs; however only colostrum was able to decrease the rates of diarrhea (Svendsen and Wilson, 1971; Wilson and Svendsen, 1971). Studies involving *transmissible gastroenteritis virus* (TGE) were the classic pioneering investigative efforts which identified the ability of previously infected sows to transfer their protection from disease to their offspring via colostrum and milk (Haelterman and Hooper, 1967). Svendsen and Wilson (1971) investigated and confirmed the hypothesis that feeding viable cultures of *E. coli* to sows prior to farrowing will control diarrhea in baby piglets (Svednse and Wilson, 1971). Kohler (1974), in one of his studies, investigated the immunologic value of mammary secretion when dams were orally exposed to *E. coli* enteropathogenic strain during the last

month of gestation. His results demonstrated that the immunologic value of colostrum can be enhanced through oral exposure of the animals with viable cultures of *E. coli*. Furthermore, animals which were exposed multiple times developed greater immunity as measured by resistance of the offspring to *E. coli*. His explanation was that natural exposure in the environment contributes to an immunological response in the dam which is subsequently transferred to the offspring via colostrum. Kohler (1974) also noted that for some strains of *E. coli*, the antigenic mass of the exposure and the frequency of the exposure highly influenced the immunological response of the dam (Kohler, 1974).

Oral Controlled Exposure Background

Oral exposure to indigenous animal origin materials has been widely used in swine production over the years to stimulate herd immunity, thus controlling and preventing diseases. The process consists of an intentional exposure of the animals with feces, tissue, or environmental materials. These presumably contain potential farm pathogens and by (re)exposing sows prior to farrowing, the objective of increasing specific antibody concentrations in the colostrum thus conveying immunity to the offspring is met. This procedure is generally used to expose pre-breeding age gilts or third trimester gestating sows and gilts. The principle of this procedure is very similar to that of the primary vaccination concept. Animals are exposed at a particular time in a controlled manner to materials containing a specific pathogen. The objective is for the animal to develop a stronger immune response to the pathogen and consequently prevent the animal or its offspring from showing clinical disease when infected later on in life. However, it is important to point out that these methods are not expected to prevent infection or shedding of the organism when encountered later, but are expected to mitigate disease expression by enhanced maternal immunity via colostrum.

A similar intervention concept which developed after oral controlled exposure (OCE) was introduced is the use of pre-farrowing vaccination with the goal of boosting the passive transfer of immunity thereby preventing disease in their offspring. Several studies have demonstrated the development of specific antibodies which could be found in serum and in

colostrum after immunization. For example, in a study conducted by Sorensen and Askaa, (1981), they demonstrated that specific antibodies for parvovirus developed after vaccination protecting the fetus from infection. Furthermore, additional studies demonstrated that the specific parvovirus antibodies could be detected in serum and colostrum from vaccinated dams as well as from serum of piglets after they ingested the colostrum (Gagrcin et al., 1990; Paul et al., 1982; Sternovsky and Zuffa, 1988). The mechanism involved in this process consists of boosting the immunity of the dam before farrowing which enhances the passive transfer of specific antibodies to the piglets. This concept is widely accepted and used in swine medicine, and is necessary due to the lack of antibody transfer from the dam to the fetus prior to farrowing. This topic will be discussed in detail below.

The oral controlled exposure procedure is referred to as “feedback” by veterinary practitioners in the field. There are different purposes for applying this technique although there are two common justifications for its use. The first purpose is part of a process called acclimation or acclimatization, usually done with gilts. Prior to entering the sow farm, young breeding females are exposed to feedback materials from sows currently housed at the unit. The primary objective of this process is to expose breeding females to the potential pathogenic microflora present in the sow farm and by doing so, enhance “herd immunity” to those potential pathogens endemic in the herd. Often, this is for control and prevention of reproductive diseases, most often porcine parvovirus. In this case the exposure will include fecal material and reproductive waste, especially mummified piglets. The theoretical goal is to expose replacement gilts prior to breeding, avoiding an inevitable infection during gestation which could harm the fetuses. . This procedure is performed during acclimatization and prior to the first breeding.

The second general purpose of oral controlled exposure is for the prevention and control of diseases in piglets. The newborn or suckling piglet diseases of interest are usually enteric caused by endemic farrowing house agents. It is also thought that some immune mediated preventive effects may extend to the post weaning period. It has become a common practice in many herds to intentionally feed farrowing house-derived materials (usually feces) to pregnant animals several weeks before farrowing. The objective is to boost the dams’

immunity pre-farrowing as well as to improve quality and quantity of passive transfer of immunity to offspring. The utility and methods of this procedure are well-documented for two classic neonatal diseases; *transmissible gastroenteritis virus* (TGE) and enteropathogenic *E. coli* (Bay, 1953; Kholer, 1974). The concepts successfully used for these agents are also thought to be applicable for other neonatal enteric diseases such as *Clostridium* and Rotavirus associated enteritis. Accurate diagnosis of infectious diseases is very important in modern swine production; however the diagnosis and control of neonatal diarrhea is complicated by interactions of infectious agents and risk factors.

Even though this may be a routine practice adopted by many swine operations, there is a lack of a standardized protocol and a lack of scientific information regarding benefits or possibly harmful effects. There is strong agreement amongst swine veterinarians that investigations comparing methods and subsequent responses need more research. Controlled oral exposure when used to control agents other than *E. coli* and TGE is a practice which needs clarification using acceptable research methods. This technique likely will be scrutinized in the court of public opinion, hence the need to clearly understand the process and benefits of this procedure are needed.

Basic Swine Immunology

There are many factors that influence resistance to disease. Some are nonspecific or innate whereas others are specifically targeted by the adaptive immune system, also known as acquired immunity. Before discussing the latter, it is useful to be generally aware of the resistance provided by the physical barriers and physical mechanism which frequently will be the first protection utilized by the host. The intact skin and epithelial barriers of the alimentary and respiratory tract provides an effective mechanism in preventing the microbial invasion. In the respiratory and gastrointestinal tracts, there are adaptations of these physical barriers that are important as well. The respiratory tract has a constant flux of proteins, mucus and water to help flush out invaders via the mucociliary escalator. This is augmented by physical defenses such as coughing and sneezing. A number of swine pathogens affect some of these mechanisms in order to establish and create disease. For instance the

colonization of the airways to *Mycoplasma hyopneumoniae* starts with the organism biding the cilia of epithelial cells (Zielinsky G C, 1992). The colonization of the cilia results in ciliostasis, a clumping and loss of cilia (DeBey and Ross, 1994). This process will ultimately result in a significant decrease in the ability of the mucociliary apparatus to function and consequently reduces the pig ability to clear the airways of debris and invading pathogens. Similarly, the gastrointestinal tract defenses include inflammation, mucus, and pH, with physical removal of offensive materials via diarrhea or vomiting.

Only a small proportion of the world`s microorganism species are associated with animals and a smaller proportion actually cause disease as primary or obligate pathogens. Still, the total number of microorganisms present in an animal is several trillions, composed of hundreds of different genus and species of microbes. In adults, the epithelial surfaces of skin and intestines have a well established normal flora which competes for ecologic niches (Artis, 2008). Pathogens and potential pathogens must compete as well and fortunately are often less adept at this task.

The immune system of swine is very similar to other vertebrates and is composed of the two major aforementioned components: innate immunity and acquired immunity. The innate immunity is a non-specific response and is very important in the early stages of the infection. The process by which the innate immune cells respond at the site of microbial invasion is called inflammation. Inflammation increases the blood flow to the site, making it possible for defensive cells and regulatory molecules to concentrate rapidly. This process consists of the activation and direct migration of immune cells from the blood stream to the affected site. The main components involved in this process are macrophages, dendritic cells (DC), natural killer cells, a variety of blood proteins (complement), a host of inflammatory mediators, and cytokines including interferon (IFN). In swine the newborn piglet has lower levels of complement activity at birth (Tizard, 2009) and it has also been demonstrated that there is a correlation between birth weights with levels of complement activity, with heavier piglets showing significantly higher concentrations of complement in their blood (Rice and L'Ecuyer, 1963).

Even though the innate immune response is very important, it lacks any form of memory therefore all infections are initially combated the same no matter how frequently the body has been exposed to a particular microorganism. In contrast to the innate response, the acquired response is antigen specific and capable of generating an immunological memory. Although acquired immunity develops slower than the initial innate reaction, the response is very effective and vital for the body's defense. When a primary exposure to a pathogen occurs, the acquired immunity can take several days to respond; however with subsequent invasion, the response occurs more rapidly due to immunological memory. The two principal components of the acquired system are the humoral and the cell-mediated effectors. The humoral immune (HI) response relies on B cells which are responsible for antibody production. B cells can be found in the cortex of lymph nodes, in the spleen, in bone marrow, throughout the intestinal mucosa in Peyer's patches, and a few are even found circulating in the blood. This humoral or antibody response is most effective against extracellular pathogens. The antibodies bind to specific antigenic sites on the pathogen which prevents attachment to cell membranes and by enhancing the processes of phagocytes and complement fixation. Both of activities will result in the destruction and elimination of the pathogen (Tizard, 2009). Antibody molecules are found in most body fluids; however serum contains the higher concentrations.

The cell mediated immune system (CMI) is comprised of T helper cells and cytotoxic T cells (Tizard, 2009). The CMI response is generally directed against intracellular pathogens that invade the host cells. The T helper cells are responsible for helping to initiate and regulate the immune response while cytotoxic T cells are responsible for killing cells which have been invaded by an intracellular pathogen.

Even though the two arms of the immune system are comprised of totally different classes of cells and present different characteristics they are fully interrelated. This interconnection is made through cytokines and antigen presenting cells which link the two arms together. Although antigen presenting cells have an important role in the innate response, they are also very important for activation of T helper cells and ultimately in directing the acquired antibody and cell-mediated response. Those cells which are rich in surface MHC class II molecules will bind the antigenic fragments that are recognizable by T helper cells. The main

cells involved in presenting antigens through this process are macrophages and dendritic cells. In swine, all components of the innate and acquired immune systems develop *in-utero* and are functional at birth although the piglet will lack immune memory and any response will be delayed and not as efficient as in adult animals (Hammerberg et al., 1989). Unless infected *in-utero,* the newborn piglet has not been exposed to foreign antigens therefore humoral and cell-mediated responses have typically not been stimulated. To illustrate, infection of pregnant sows with porcine parvovirus in the first half of gestation results in embryonic or fetal death followed by re-absorption or mummification. However if the infection occurs later than 70 days of gestation, fetuses will likely become immune and survive without any clinical evidence of the disease. Thus, pig fetuses are immuno-competent for porcine parvovirus after the 70 days of gestation and the piglets are able to develop protective immunity in response to the virus (Bachmann et al., 1975). Numerous studies indicate fetuses are able to produce antibody in response to various types of antigenic stimulation when infected in late gestation (Bachmann et al., 1975; Cutlip and Mengeling, 1975; Mengeling, 1975; Redman et al., 1974).

Immunological Review of Swine Gastrointestinal Tract

Histologically the gastrointestinal tract has four distinct functional layers; mucosa, submucosa, muscularis propria and adventitia. The mucosa is composed of epithelium, lamina propria and a muscularis mucosa. The submucosa is formed by a loose collagen layer, large blood vessels, lymphatics and nerves. The tunica muscularis consists of smooth muscle arranged as an inner circular layer. Lastly, the adventitia is composed of loose supporting tissue to major vessels and nerves.

The surface of the intestine is vast. In humans there is approximately 100 m^2 of surface area (Artis, 2008). The intestinal mucosa is lined by a single layer of columnar epithelial cells that form a barrier between the intestinal lumen and the host connective tissue. The mucosa is constantly bathed with commensal and pathogenic microorganisms. Commensal microorganisms normally live in a symbiotic relationship with the animal. For example, it is estimated that human intestine is home to approximately 10^{14} commensal bacteria (Ley,

2006) and between 500-1000 different species. This concentration and diversity of bacteria has been described as one of the most densely populated microbial habitats known in biology (Gill, 2006). In response to the remarkable and continuous immunological challenges, the gastrointestinal tract (GIT) has developed a vast and complicated immunological apparatus. The intestines contain a population greater than 10^{14} lymphocytes and have the greatest concentration of immunoglobulins compared to other body systems (Mayer, 2000).

The immune cells located in the intestine have the capability to recognize and tolerate non-invasive commensal, remaining hyporesponsive to them while at the same time possessing the ability to generate an immune response when pathogenic microorganisms are present. The mucosal immune complex is equipped with gut-associated lymphoid tissue (GALT) which mediates the adaptive immune responses. Immune cells are well organized within GALT providing a specific and efficacious defense. This region contains the largest collection of immune cells in the body (Mowat, 1997) and this is the sites where B and T lymphocytes interact with gut associated antigens. Here, antigens are constantly sampled in the gut lumen and then presented to the GALT lymphocytes, generating a multitude of immune responses.

The mucosal epithelium cells play a very important role in the immune response. They provide intrinsic and extrinsic mechanisms which protect the host. The tightly organized intestinal epithelial cell (IEC) junctions form an effective physical barrier against microorganisms (Artis, 2008; Burkey, 2009) as well as facilitating the presentation and transfer of potential antigens to the cells located in the GALT. These two mechanisms are part of the intrinsic mechanism protecting the host. Extrinsically, the IEC secretes a wide range of antimicrobial substances such as peptides, mucins, and immunoglobulins. Both intrinsic and extrinsic mechanisms are key factors in preventing the entry of commensal and pathogenic bacteria (Artis, 2008). These barriers limit the interaction of pathogenic agents with the cellular mucosa (Oswald, 2006).

Luminal contents are constantly sampled by specialized lymphoid structures known as Peyer's Patchers which are located throughout portions of the small intestine. Isolated

lymphoid follicles located along the intestine's lamina propria are also active in antigen sampling. Antigens are sampled by phagocytic microfold cells (M-cells), dendritic cells and/or by intestinal epithelial cells. M-cells are found in the follicle-associated epithelium of Peyer's patches, and are responsible for sampling luminal antigens and microorganisms; the sampling occurs by active transport across the epithelium and delivery to specialized antigen-presenting cells such as dendritic cells (Bailey, 2009). The efficacy with which the dendritic cells (DC) acquire antigens from the lumen is well established and described (Chirdo, 2005; Milling, 2005). The process occurs when DC insert their dendrites between the tight junctions of the IEC and reach the intestinal lumen (Rescigno, 2001).

The IEC is also capable of recognizing bacteria and some of their products through structures called toll-like receptors (Takeda and Akira, 2003) and nucleotide-binding oligomerization domain-like receptors (Meylan, 2006). Detection of bacteria and/or bacterial products by toll-like and other receptors activates a cascade of signals which ultimately result in the release of proinflammatory cytokines (Ghosh et al., 1998). Some antigens are capable of crossing the intestinal lumen and will interact directly with macrophages, dendritic cells, and B and T lymphocytes in the GALT (Burkey, 2009). After antigens have been internalized within dendritic cells and macrophages they are processed. The processing consists of breaking the antigen molecules into smaller peptides which can then be bound to the major histocompatibility complex (MHC) molecules. These are specialized antigen presenting receptors located on the cell surface and when a MHC-bound antigenic peptide binds to a T lymphocytes receptor an acquired immune response is initiated.

Swine Placenta Physiology and Colostrum

In primates and rodents, a considerable transfer of antibodies from the maternal circulation to the fetus occurs prior to birth. In contrast, the transfer of antibodies across the placenta is not observed in domestic species including cattle, sheep and swine. The histological structure of the placental interface will influence the nature of molecular transport across the placenta. In swine, the placenta is composed of six layers of distinct cells therefore the fetus is separated from the dam's blood supply during the intrauterine period (Hermann, 2009).The concept of

vaccination pre-farrowing is extensively used in swine production however the results are highly dependent on colostrum ingestion by the newborn piglet. There is no transfer of antibody or immune cells across the placenta in swine.

The first report discussing the implications of this characteristic in swine was made by Hayes (1921) when he observed that piglets born from sows infected with *Brucella abortus* did not possess any protection (agglutinins) against the pathogen however the protection was present in piglets after nursing the same sows. Nelson (1932) conducted a study where sows were either immunized against vaccinia virus prior to or shortly after breeding. After farrowing some of the piglets were not allowed to nurse preventing colostrum ingestion. These were fed an alternative feed source containing a mixture of commercial cow milk powder and swine serum. Seven days after farrowing, piglets were challenged with vaccinia virus and monitored for 10 days post challenge for vaccinia infection. The results demonstrated that piglets which had nursed the immune sows did not develop disease (Nelson, 1932). However piglets that were not allowed to nurse and piglets from non-vaccinated sows developed a typical vesicular reaction due to the infection. The results from this study strongly support the conclusions drawn by Hayes in his 1921 study. Both authors concluded that the swine placenta was not permeable to the transfer of protection and that the passive protection in swine was mainly achieved through the ingestion of colostrum. Simply put, the swine placenta does not allow the transfer of antibodies from the dam to the fetus. Pigs have an epitheliochorial placenta which is impermeable to immunoglobulins therefore piglets are normally hypogammaglobulinemic or agammaglobulinemic at birth (Kim, 1975).

Piglets are born virtually sterile from microbial flora and with low energy reserves and lacking pre-parturition immune responses. The newborn piglet faces serious challenges at birth, as the new environment immediately exposes them to a host of microbial pathogens and shockingly cooler temperatures. The energy requirement of the neonatal piglet is quite high and vital for survival (Le Dividich et al., 2005). The animal is in a fast growth stage and energy is extremely important for maintenance, thermoregulation, growth and physical activity. The nutritional requirements are maximal at birth when expressed on a body-weight (BW) basis (Le Dividich et al., 2005) In this study Le Dividich estimated that under

conditions of thermal neutrality, minimum energy expenditure associated with feeding (i.e. tube- or bottle-feeding) and physical activity, the energy required for maintenance is 275 kJ/kg BW (Le Dividich et al., 1994). However the reality in commercial situations is that piglets face significant cold stress challenges and therefore extra energy is required for thermoregulation. This extra energy averages 2 kJ/kg/h/^{o}C below the critical temperature (Le Dividich et al., 1998).

There are three major components of the body which can potentially be used to provide an immediate source of energy at birth. These are body reserves of protein, glycogen and fat. Newborn piglets are poorly insulated and lack brown adipose tissue and body protein accounts for a only a small proportion of immediate thermoneutral heat production due to the low catabolic rate at birth. It is estimated that the total body reserves of glycogen vary from 30–38 g/kg birth weight at parturition (Le Dividich et al., 1994). In normal environmental conditions, 75% of liver glycogen and 41% of muscle glycogen are utilized by the piglet within 12 hours postpartum (Elliot and Lodge, 1977). Thus the glycogen reserves can be rapidly depleted. Cold stress can speed the process of depletion. In addition, recent market trends to select pigs with reduced carcass fatness have resulted in leaner pigs at birth (Herpin et al., 1993) with lighter livers and less liver glycogen (Canario et al., 2005). The extra energy required by the newborn piglets is met through the ingestion of colostrum. Colostrum is defined as the first secretion of the mammary gland. Colostrum is composed of approximately 16mg/ml of crude protein, approximately 4mg/ml of lactose and lipid (Le Dividich et al., 2005). The mean concentration of protein in colostrum is 100mg/g (Klobasa and Butler, 1987), and the amount of total protein exported in the first 24 hours via colostrum varies between 260 to 600g. Fats are the main source of energy in colostrum and account for 40% to 60% of the total energy available (Le Dividich et al., 2005).

Colostrum intake by piglets is influenced by a combination of factors including the ability and vigor of piglets to quickly locate a teat and suckle and also the ability of the sow to produce sufficient colostrum to satisfy the litters' necessity (Devillers et al., 2007; Hoy et al., 1995). The colostrum yield and the IgG concentrations vary considerably among sows (Inoue et al., 1980; Klobasa and Butler, 1987). The colostrum production in sows can be accessed

through the increase of litter weight between birth and the first 24 hours while accounting for piglets' excreta (Devillers et al., 2004). Several studies have investigated the difference in weight gain of piglets in the first 24 hours (Le Dividich et al., 2004; Pattinson and Thomas, 2004; Pattinson et al., 1995; Thompson and Fraser, 1988) with the unanimous conclusion that colostrum production is highly variable. Theoretically, factors such as health, nutrition, parity, genetics, or change in hormones could heavily influence this variability. However, more research is needed to fully understand the factors affecting the production and composition of colostrum (Farmer and Quesnel, 2009).

Even though neonatal piglets are able to produce an immune response to antigens by parenteral (Binns, 1967) and enteric routes (Redman et al., 1978), their immune system is underdeveloped at birth and remains so until weaning. Therefore survival of neonatal piglets is highly dependent on the timing, quality and quantity of colostrum ingestion. (Hoy et al., 1995; Tuchscherer et al., 2000). For instance, a positive correlation has been demonstrated between the concentration of IgG in piglets' plasma shortly after birth and survivability (Hendrix et al., 1976). Furthermore, studies comparing dead piglets with surviving counterparts found a lower concentration of IgG in the plasma of dead piglets (Drew and Owen, 1988; Klobasa et al., 1981).

Colostrum and milk have different compositions, especially when immunoglobulin is taken into consideration. The colostrum antibody concentration is largely composed of IgG and the estimated ratio of IgA to IgG ranges from 0.16 to 0.22. This ratio is 2.1 to 6.96 in milk (Berthon et al., 2000). Bourne and Curtis (1973) used radioactive labeling of immunoglobulin to demonstrate that nearly 100% of colostral IgG, 40% IgA and 85% of IgM are derived directly from the sows' serum. By contrast, 70% of the IgG, 90% of the IgM and 90% of the IgA found in milk are synthesized locally at the mammary gland (Stokes and Bourne, 1989). According to Neville et al. (2001), colostrum synthesis in the mammary gland starts before the parturition. The tight junctions between secretory cells of the mammary glands are open during the last month of gestation which enables the massive sequestering of antibodies from the serum into the colostrum (Neville et al., 2001). The closure of the junctions occurs between 24 and 36 hours after the beginning of farrowing

under hormonal control by progesterone, cortisol and prolactin (Neville et al., 2001; Nguyen et al., 2001). This process in part, explains the rapid change in immunoglobulin constituents between colostrum and milk.

The outcome of oral controlled exposure is directly linked to colostrum intake and stimulation of antibody production in the sows. Corresponding to the antibody class switch in milk, gut closure in the piglet occurs about 24 to 36 hours after birth blocking the absorption of large macromolecules (Lecce, 1973). Furthermore, the concentration of immunoglobulins in colostrum dramatically declines within the first few hours after farrowing (Bourne, 1969; Klobasa and Butler, 1987).

In a study where sows were hyperimmunized with *E. coli* it was reported that piglets were born practically free of antibodies however high amounts of *E. coli* specific antibody was detected after colostrum ingestion. This observation has been repeated several times in the literature. Moreover, this study also concluded that the ability of the baby pigs to absorb intact antibodies decreased 50% every 3.06 hours from time of parturition initiation to the first day of age (Speer, 1957). Therefore the timing and quantity of colostrum intake plays an important role in the outcome of the oral controlled exposure and subsequently on the piglet's health (Passille and Rushen, 1989; Tyler et al., 1990).

Clostridium Perfringens

Clostridium perfringens was first described in 1891 by Alchame as the Bacillus of Acute Rheumatism. Since then, it has received different names such as *Bacillus phlegmoni, Bacillus cadaveris butyricus, Bacillus aerogenes capsulatus, etc.* (Odendaal et al., 1994). *Clostridium perfringens* is gram positive, endospore forming and anaerobic. *Clostridium perfringens* (*Cp*) may be the most widely occurring pathogenic bacterium (Smith and Willians, 1984) and is certainly the most important cause of clostridial enteric disease in domestic animals (Songer, 1996). It is widely spread in the environment occurring in soil, sewage and water, as well as in the intestinal tract of humans and warm blooded animals (Odendaal et al., 1994). The species is divided into five types based on the production of the four major toxins: alpha, beta, epsilon and iota. *Clostridium perfringens* type A (*CptA*) are

consistently recovered from the intestinal tracts of animals and from the environment while other types, B, C, D and E, are less likely to be recovered from the intestinal tracts of animals (Carter and Chengappa, 1991) but can be found in the environment in areas where associated disease is enzootic (Niilo, 1980).

Clostridium perfringens type A (*CptA*) and type C (*CptC*) are the most important enteric clostridial pathogens in swine. *Clostridium perfringens* type C elaborates alpha toxin and beta toxin as major toxins while *CptA* has only the alpha toxin. Others toxins may be elaborated as well. Recent studies have shown a strong correlation among pigs with clinical signs of diarrhea and the presence of a *CptA* which also produce a beta 2 toxin. Studies have shown that only a few isolates recovered from normal pigs contain genes for beta 2 toxin production while over 90% of the isolates recovered from *Cp* associated porcine neonatal diarrhea are positive for beta 2 toxin gene (Bueschel et al., 2003). More research is necessary to better understand the role of the beta 2 toxin in pathogenesis of disease. In a recent survey study performed at Iowa State University, it was reported that 48% of all submissions between March, 2005 and March, 2006 of pigs less than 10 days old with a complaint of diarrhea were diagnosed with *CptA* and that *CptC* was only rarely diagnosed (Yaeger, 2007). This disparity may be explained by the fact that the vaccine for *CptC* has been available for many years, is widely used and highly effective**,** while the vaccine for *CptA* is relatively new and the efficacy is not established and often appears variable.

The main source of transmission of *Cp* is piglet-to-piglet however it is believed that the intestine of sows might be the origin source (Songer, 1996). *Clostridium perfringens* type C is not part of the normal flora of the swine intestine, which may explain the sporadic nature of disease. Screening of replacement animals through culturing or by PCR methods of swine feces is not a feasible option for *CptC* because its presence can only be found in a very small percentage of positive animals. This makes detection of *CptC* unlikely by current diagnostics methods. The use of more sophisticated and costly diagnostic methods are not feasible for large numbers of animals. Likewise, *CptA* screening is also not feasible since *CptA* is part of the normal flora in the swine intestine (Mansson and Smith, 1962). The disease caused by either type *CptA* or *CptC* is commonly seen in the first 3 days of life. However, it may appear

as early as 12 hours after birth (Bergeland et al., 1966; Meszaros and Pesti, 1965). There are two characteristics peculiar to neonatal pigs which may account for a higher susceptibility compared to other neonatal groups of animals. The first one is the trypsin secretion deficiencies in piglets and the second one is the presence of colostral protease inhibitors, both of which impede the ability to degrade the *Cp* protein toxins.

Clostridium perfringens type C occurs epizootically in non-vaccinated herds (Bergeland, 1966). In affected litters the prevalence can reach 100% and the mortality varies depending of the immune status of the sows. The total mortality can be as high as 50% to 60% among affected litters but mortality of 100% is not uncommon in piglets born by non-immune sows (Bergeland, 1966; Hogh, 1967). Affected piglets usually develop hemorrhagic diarrhea, dehydration and rapidly loose body condition. Peracutely affected piglets often die within 12 to 36 hours after parturition. At necropsy, diffuse hemorrhages may be found in the small intestines with focal areas of necrosis in the jejunum and/or ileum. The mucosa is reddish with intense hemorrhage and gas bubbles within the small intestine wall. In less acutely affected piglets it is possible to observe a reddish-brown diarrhea, some level of dehydration and loss of body condition. Piglets may survive for 1 or 2 days after the manifestation of clinical signs but most often die within a few days of onset. At necropsy lesions may be more segmental with necrosis, hemorrhage and emphysema in specific but limited portions of jejunum. The intestinal wall is often thickened and yellow or grayish (Songer and Taylor, 2006). In contrast to peracute and acute scenarios, subacute cases of the disease do not develop a hemorrhagic diarrhea but often develop a yellowish diarrhea with some flecks of necrotic debris. Subacute piglets loose condition gradually over several days and become thin and dehydrated eventually dying about 5 to 7 days after birth. At necropsy the intestinal wall is covered by a closely adherent necrotic membrane and the wall is usually thickened and friable. In more chronic cases of the disease piglets have intermittent diarrhea for a week or more and feces are typically yellow-gray and mucoid. Chronic piglets typically retain their normal behaviors but many may eventually die several weeks later due to a continual loss of condition. The attachment of the organism most often occurs with jejunal epithelia cells at the villus apices (Arbuckle, 1972; Walker et al., 1980a). This process of attachment is

followed by desquamation of cells and proliferation of the organism. This process results in necrosis of the villus lamina propria and hemorrhage. Bacteria can remain on the necrotic villi or be shed in the hemorrhagic diarrhea as vegetative bacterium or spores (Kubo and Watase, 1985). Beta toxin is the main factor involved in the pathogenesis of the disease associated with *CptC* (Hogh, 1967; Warrack, 1963).

The diagnostic history of the infection in a herd, the clinical signs and the gross and microscopic lesions associated with *CptC* are enough information for a presumptive diagnosis of the disease. In circumstances where more diagnostic confirmation is needed the use of PCR methods for toxin detection (Buogo et al., 1995; Meer and Songer, 1997; Songer and Meer, 1996) and enzyme immunoassays are useful tools to confirmatory diagnostic. Treatment is seldom used and of little value with clinically affected animals (Hogh, 1967; Szabo and Szent Ivanyi, 1957). To protect non-immune litters facing risk of an outbreak, the use of equine antitoxin can be administered and the resulting passive immunity can last as long as 3 weeks (Ripley and Gush, 1983). Some antibiotics such as penicillin, ceftiofur, and bacitracin methylene disalicylate can be used metaphylatically for treatment and control of the disease. Although these methods are available to control and prevent the disease, the most accepted method of prevention is achieved by vaccination of sows with a *CptC* toxoid at approximately 2 to 3 weeks before farrowing (Kennedy et al., 1977a).

Piglets affected with *CptA* often develop a pasty cream colored diarrhea with fecal staining of the perineum and gradual loss of body condition with a rough hair coat (Arbuckle, 1972; Johannsen et al., 1993a). The mortality is very low with most piglets recovering within 3 to 5 days. Recovered pigs are often unthrifty and this sometimes affects their life time growth performance (Johannsen et al., 1993b). At necropsy the small intestine is typically flaccid, thin walled and usually filled with of gas and watery contents. At the microscopic level, there may be a superficial villus tip necrosis and accumulation of fibrin. However, it is not uncommon for the intestines to appear morphologically normal. The jejunum and ileum may be heavily colonized with *Cp* (Nabuurs et al., 1983). In most naturally occurring cases the absence of gross and microscopic lesions implies that the enteritis is essentially a secretory diarrhea. The mechanism by which the toxins may cause the disease is not completely

understood.

The *CptA* epidemiology and pathogenesis mechanisms remain unclear at this time, thus a diagnosis is most often made by the clinical signs, isolation of a large amount of organism from the affected jejunum and ileum and through the process of ruling out the participation of other known pathogens. Genotyping of *Cp* isolates can be performed and the results usually reveal a type A possessing the gene for beta 2 toxin production. The treatment of *CptA* with antibiotics has variable success. Another option is the use of a toxoid vaccine in pregnant sows several weeks before farrowing. However commercial vaccines do not include the beta 2 toxin. The development of an efficacious vaccine is needed but complicated since the roles of the toxins responsible for the clinical disease have not been fully elucidated.

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CHAPTER 3. METHODS FOR CHARACTERIZING ORAL CONTROL EXPOSURE IN SWINE FROM FIELD SETTINGS

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Abstract

Oral exposure to herd-derived animal materials has been widely used in swine production over the years as a way to enhance herd immunity as a way of controlling and preventing diseases. The process consists of intentional animal exposure to feces, tissues, or environmental materials, presumably containing potential pathogens, with the objective increasing immunity thus decreasing clinical disease. The process is generally known as feedback in the field. Due to the lack of scientific information, the objective of this study was to better characterize the oral controlled exposure (OCE) methodology and to determine if the OCE process would increase the quality of colostrum. The study population was comprised of 40 gilts pre-farrowing receiving different OCE levels and a control group. Measurements such as total IgG, white cell count, and antibodies for clostridiums toxins were individually accessed in colostrum. Feces from animals at farrowing were accessed to determine possible microorganism shedding trends. The results demonstrated no statistical differences among groups when considering the immunity parameters however a numerically trend is observed when antibodies for alpha toxin were analyzed. *Clostridium perfringens* and coliforms shedding at farrowing were not influenced by the OCE protocol.

Introduction

Oral exposure to herd-derived animal materials has been widely used in swine production over the years as a way to enhance herd immunity for control and prevention of endemic diseases. The process consists of intentional animal exposure to feces, tissues, or environmental materials, presumably containing potential pathogens, with the objective increasing immunity thus decreasing clinical disease. In 1953, Bay concluded that sows fed infective intestinal material with a coronavirus (transmissible gastro enteritis virus), at various intervals before farrowing were capable of transferring immunity to their offspring (Bay et al., 1953). In 1974, Kohler`s studies demonstrated that feeding back enteropathogenic *E. coli* to sows during the last month of gestation increased the anti-diarrheagenic value of colostrum and milk (Kohler, 1974). However, it is important to note that these methods are not expected to prevent infection or organism shedding but to provide protective immunity.

Accurate neonatal diarrhea diagnosis and control is increasingly complicated by infectious agent interactions and numerous risk factors. Multifactorial disease does not respond well to a single intervention. Variation in organism strain, production practices make it difficult to have uniform exposure hence one method to achieve a uniform immunity to a microflora in the herd is intervention with feedback. Intentional farrowing house-derived materials (usually feces) feedback is provide to pregnant animals five to three weeks before farrowing with the objective to bolster both the dam`s immunity and passive protective antibodies transfer to offspring. This concept is particularly adaptable to swine because the newborns piglets depend on colostrum since the porcine placenta does not permit antibody transfer and modern production practices have limited the dam’s access to herd waste materials which are likely to contain endemic pathogens. Pigs have an epitheliochorial placenta which is impermeable to immunoglobulins; therefore, piglets are hypo or agammaglobulinemic at birth (Kim, 1975). Neonate piglets are able to produce an immune response to antigens encountered by either parenteral (Binns, 1967) or enteric routes (Redman et al., 1978) but their immune system is immunologically underdeveloped at birth, continuing to mature until weaning. Therefore the piglet survival at birth depends on colostrum ingestion timing as well as the antibodies and immune cells produced by the dam and delivered through the colostrum

(Tuchscherer et al., 2000). Antibodies and colostrum immune components are determined by through antigenic stimulation of the dams' systemic immune system (Salmon et al., 2009). Colostrum and milk have different immunoglobulin concentrations and types. Colostrum is comprised of IgG; the estimated IgA to IgG ratio ranges from 0.16 to 0.22 in colostrum and 2.1 to 6.96 in milk (Berthon et al., 2000). Bourne and Curtis (1973) reported through radioactive labeling of immunoglobulins, that in colostrum 100% of IgG, 40% IgA and 85% of IgM are derived from sow serum. According to Neville and others (2001), colostrum synthesis in the mammary gland starts before parturition. The tight junctions between secretory cells of the mammary glands are open during the last month of gestation which enables the massive antibodies sequestering of from the serum into the colostrum. The junction closure occurs between 24 and 36 hours after the beginning of parturition under progesterone, cortisol and prolactin hormonal control (Neville et al., 2001; Nguyen et al., 2001). Furthermore, the antibodies sequestering, especially IgG and IgM, is actively mediated by specific receptors on the mammary epithelial cells (Huang, 1992). This process explains in part the dramatic difference in immunoglobulin concentrations between colostrum and milk.

Swine colostrum also contains additional components important to immunity, including polymorphonuclear cells, lymphocytes, macrophages and epithelial cells. The quantity of each cell type varies according to physiologic stage as well as the condition of the animal (Salmon et al., 2009). The colostrum cellular components are largely composed of phagocytic cells while milk contains mostly epithelial cells. In post colostral sow milk, epithelial cells account for approximately 31% of total cells, lymphocytes account for about 10% (Lee et al., 1983; Magnusson et al., 1991), neutrophils for 47%, eosinophils 1% and macrophages 9% (Schollenberger et al., 1986a; Schollenberger et al., 1986b). In colostrum, lymphocytes account for approximately 26% of total cells, where the majority are T cells and about 30% are B cells (Schollenberger et al., 1986a; Schollenberger et al., 1986b). These leucocytes are readily absorbed through the intestine villi into the circulatory system of the neonate pig (Tuboly et al., 1988) and transported to mesenteric lymph nodes and other tissues where they exert an immuno stimulant effect in response to antigens (Williams, 1993). The

impact of timing of vaccination and exposure for optimizing colostral antibodies has not been studied.

Another factor influencing the successful outcome of the oral controlled exposure (OCE) is directly related to colostrum intake by piglets. In piglets, gut closure occurs about 24 to 36 hours after birth which means the concentration of large macromolecules absorption is discontinued (Lecce, 1973). Furthermore, immunoglobulin concentration in the colostrum dramatically declines within the first few hours after farrowing (Bourne, 1969; Klobasa and Butler, 1987). Therefore, colostrum intake timing plays an important role in neonatal piglet health and early survivability (De Passille´ and Rushen, 1989; Tyler et al., 1990).

Clostridium perfringens (*Cp*) is a gram positive, endospore-forming and anaerobic bacterium. The two main *Cp* types causing diarrhea in pigs are *Clostridium perfringens* type A (*CptA*) and *Clostridium perfringens* type C (CptC). The *Cp* classification is based on the specific toxins produced. *CptC* expresses both alpha and beta as major toxins whereas *CptA* makes alpha and beta 2 as its major toxins. Both types of *Cp* are well-recognized as diarrhea causes in neonatal piglets. In survey study (March 2005- March 2006) performed at Iowa State University, Yaeger (2007) reported that 48% of all diarrhea causes in pigs less than 10 days old were diagnosed as *Cp*tA (Yaeger, 2007) while *CptC* was rarely diagnosed. Vaccine for *CptC* has been available for many years and is widely used and generally effective**,** whereas the vaccine for *CptA* is relatively new and the efficacy is questionable. *Clostridium perfringens* type A disease is commonly observed within the first 3 days of life and it may appear as early as 12 hours after birth (Bergeland et al., 1966; De Passille´ and Rushen, 1989; Matthias et al., 1968; Meszaros and Pesti, 1965; Tyler et al., 1990).

The epidemiology and accurate diagnosis are often unclear, however it is believed that sow vaccination pre-farrow and good sanitation protocols will reduce or eliminate clinical disease associated with this agent. The pre-farrow vaccination will play a role on the immunity of the dam thereby augmenting the passive transfer of specific *Cp* immunity to the offspring.

Two main factors are thought to play a role in neonatal *Cp* associated disease: 1. The exposure dose of *Cp* and 2. The maternal immunity level delivery through colostrum. For the

first, sanitation protocols play an important role in reducing neonatal clinical disease by minimizing exposure to *Cp* during the critical first two days after birth. Although OCE has been used to control neonatal scours, there is increasing debate associated with the practice influence on the *Cp* shedding and coliforms by the sow at farrowing. However, there is a lack of research at this time to scientifically support this theory. In a survey conducted by Baker (2006), 44 veterinarians who were involved in diagnosing cases diarrhea caused by *Cp* were interviewed to determine the most frequently used control method. Oral controlled exposure (feedback) was the most frequently used method according to this report, occurring in about 68% of the cases (Baker, 2006). However, he also stated that veterinarians differed in their opinions of its effectiveness.

The objectives of this study were to characterize the OCE methodology, determine the OCE optimal timing as well as determine if biological parameters such as IgG, white cell or specific antibodies are suitable to monitor OCE efficacy process. The microorganism shedding was also accessed to verify the possible association.

Materials and Methods

Animals and Animal Care

The trial was performed under field conditions at a 5,400 sow farrow-to-wean commercial operation with a history of *Cp*-associated neonatal diarrhea. This operation was in the process of refining and standardizing their feedback protocol while looking for a method to measure the outcome. The farm was located in northeastern Iowa and was considered free of PRRSV at the time of the trial. Forty gilts were individually identified and randomly assigned (several random number iterations in Microsoft Excel) to four different treatment groups. The forty animals were selected according to predicted farrowing dates. Pregnancy was confirmed by real-time examination before the animals were enrolled in the study. All gilts included in the study were enrolled approximately five weeks prior to farrowing and expected to farrow within a five day period. All study animals were vaccinated (*ProSystem®CE, Intervet Inc., Millsboro, DE*) according to the current farm vaccination protocol which includes *E. coli* and *CptC* at five and three weeks prior to expected farrowing.

Gilts were housed in individual crates during gestation according to the farrowing date and moved to individual farrowing crates between estimated days 111 and 113 days of gestation. They were fed once daily and water was available *ad libitum*. The gestation barns were managed under the typical industry standard fashion as continuous flow; however, the farrowing rooms were managed on an all-in, all-out schedule. The farrowing facilities were divided into 15 farrowing rooms with 60 farrowing crates each. As a current farm management, gestating animals were routinely exposed to feces obtained from the farrowing rooms using a standardized feedback protocol. The animals were exposed to piglets' feces three times a week at five, four and three weeks prior to farrowing. However gilts enrolled in the study were not submitted to the farm feedback protocol. The gilts were closely monitored throughout the study period, and records were maintained and alteration of behavior (off feed event or illness) or any needed treatment (antibiotic, supportive) that could have an influence on the outcome to be measured was recorded. At farrowing, animals were observed every 30 minutes for parturition signs including restlessness, milk let down, and nest- building behavior, which are indicators commonly associated with the immediate pre-farrowing period (Cronin, 1991)

Study Groups

The experimental design was composed of four experimental groups: three different of oral control exposure protocols and a negative control group. Animals were the experimental unit. Group 1 (n = 10) received the oral controlled exposure according to *PIC Feedback Protocol for Female Acclimation and Gestation* (PIC, 2009) which recommends the OCE be applied at three times a week of weeks five, four and three prior to farrowing. Group 2 (n = 10) received the treatment three times a week beginning at week five before farrowing. Group 3 (n = 10) received OCE three times a week, beginning two weeks prior to farrowing. Lastly, gilts in the negative control Group 4 (n = 10) did not receive OCE. All animals were randomly assigned to treatment groups.

Sample Collection

Blood samples and fecal samples were collected from all 40 pregnant gilts prior to the start of the OCE treatment and approximately 6 weeks prior to farrowing. Feces were collected

individually, directly from the rectum of each gilt. At farrowing, fecal samples and colostrum were collected from all gilts. At least 40 ml of colostrum was collected before the first piglet nursed. Colostrum collection was manual across all sows` teats by the same individual for consistency. All piglets born by the study gilts (approximately 400) were individually identified and weighed at birth and again at weaning. All personnel involved with the data collection were blinded to the treatment groups. Performance recordings of the study gilts and their piglets were kept on the farrowing cards which were recorded at the study conclusion for subsequent evaluation.

Inoculum Preparation

Feces were collected, thoroughly mixed, and then frozen in aliquots to be used throughout the experiment (same batch for all animals). Diagnostic tests were performed weekly on the material to verifying feedback content, what was actually fed back to the gilts; for research proposes it was essential to assure consistency of the material throughout the different weeks of the experiment. The material used for OCE was comprised of piglets' feces and distilled water. Three days prior to trial initiation, the farm was visited by the research team and the fecal seed material was collected. This material was used to make the OCE aliquots which were used for all entire experiment. The collection was accomplished using a sponge and a container with water, mimicking the way it is currently accomplished in the field setting. The fecal collection procedure targeted piglets in their first two days of life, primarily those suffering from diarrhea which had not been medicated treated in any way. However, feces from older piglets were also collected if they were diarrheic. The fecal material collected for the study did not include adult feces nor was any tissue or material from dead piglets utilized. Selected farrowing crates were thoroughly wiped with a sponge which was continually rinsed in a container with a minimal amount of distilled water to maximize feedback slurry concentration. The process was repeated at least for 30 different crates which contained diarrheic piglets. Any piglet observed with diarrhea was carefully handled to allow the feces from that animal to flow directly into the collection container. The process was repeated for 3 consecutive days with the objective of collecting the ideal samples including collecting sufficient quantity quality fecal material. The total material was then allocated to a larger

container, thoroughly mixed, and then frozen in aliquots to be used *per* administration to the gilts in treatment Groups 1-3. Once a homogeneous solution was obtained, it was randomly placed into 160 of 50ml centrifuge tubes, each containing 45ml of the solution. The tubes were placed in a chest freezer at -20°C and kept throughout the experiment. Previous to this trial, fecal samples were collected and frozen for various periods of time to verify the survivability of the several specific microorganisms to determine the effect of freezing.

Inoculum Administration

Each tube was completely thawed and mixed with quarter pound of feed then provided as a mixture to each experimental treatment gilt at the specified time according to the experimental design. The 45 ml of feedback solution was dispensed in a 240 ml cup, with water just prior to administration.

Colostrum Testing

Skim milk was prepared by centrifugation of the whole colostrum sample at 1,500 X g, for 40 min, at 26C. The cream layer was carefully removed, and the whey was collected and used to perform serologic tests and the cell sediment was then used to determine white cell count. Single radial immunodiffusion (Fahey and Mckelvey, 1965) was employed in order to determine the quantity of IgG in colostrum (The *Porcine IgG RID Kit,* VMRD, Inc. Pullman, Washington). The colostrum was diluted at 1:8 with distilled water due to the assay range limitations.

Individual whey was used to perform the ELISA kit (*Bio-X Diagnostics. Jemelle, Belgium*) which is composed of a 96-well microplate previously sensitized with *Cp* alpha toxin. The optical densities in the microwells were accessed using a plate reader and a 450nm filter (*Molecular Devices Emax. SunnyVale, CA*). The percent inhibition was then calculated for each sample. Toxin neutralization test were performed to estimate the antibodies levels against *Cp* alpha toxin and antibodies against *C.difficle* toxin A and B.

Cell sediment was used to estimate the white cell in colostrum (Reber et al., 2008).The procedure was achieved by adding 1ml of Sterile Earle's Balanced Salt Solution (EBSS) to pellet in a microcentrifuge tube to re-suspend the cells, take 10ul of suspension and place in

clean microcentrifuge tube. It was added 10ul 0.4% trypan blue to suspension and later 10ul of this stained suspension was sampled and placed into a hemacytometer (Reber et al., 2008). The cells count was performed in the 1mm center square and four 1mm corner squares.

Bacteriology and Flotation

For Individual fecal samples were diluted in distilled water as follows: 125 mg (1.25ml) of feces to eight ml of distilled water. After the dilution, the solution was vortexed for approximately one minute, 50µL of the solution was pipette (*Rainin. Oakland, CA*) and plated on bacterial growth medium of Trypticase Soy with 5% sheep Blood (*Remel – Lenexa, KS*) and 50µL were plated on MacConkey agar (*Remel – Lenexa, KS*). All samples were streaked for isolation in quadrant pattern and incubated at 35C. The MacConkey plates were incubated aerobically for 24 hours and the anaerobic blood agar were incubated anaerobically (*BD GasPak™ EZ Anaerobic contain system*) for 48 hours. MacConkey plates were viewed for red (lactose positive) colonies and the anaerobic blood agar plates were viewed for double zone large colonies which stained as large gram positive rods. The growth count was assessed visually and scored from 0 to 4 according to the guideline previously established. 0 – no growth; 1- low growth, only the first quadrant; 2- moderate growth, first and second quadrants; 3- moderate to heavy growth, first second and third quadrants; 4- heavy growth, all four quadrants (Flowers et al., 2007). Fecal flotation was also performed according to the Modified Wisconsin Sugar Centrifugal-Flotation Method (Dryden et al., 2005) in order to detect oocysts of *Isospora*, *Eimeria* as well as nematode ova.

Statistical Methods

Diagnostic results associated with the variables total IgG, cellular count and anti-alpha toxin titers were defined as continuous variable and were analyzed by non-parametric procedure in SAS (SAS Institute Inc, Cary, North Carolina). The exact *P* values were computed by Kruskal-Wallis test with the objective of determining whether or not differences existed between treatment groups. Analysis of the semi-quantitative culture growth of *Cp* and coliforms was performed using a generalized linear model (GLIMMIX procedure, SAS Inst. Inc). Prior the statistical analysis, the GLIMMIX procedure used a log-transformation to transform the data which was approximated a Poisson distribution. Amount of *Cp* and

coliform shedding were analyzed using a model that included treatment as a fixed effect and gilts was treated as a random effect. The correspondent *P* values obtained were used as results.

Results

Oral Bacterial culture of the homogenized material used for feedback yielded a moderate to heavy growth of *Cp* and a moderate growth of coliforms. It was tested for Rotavirus type A in an ELISA test (Benfield et al., 1984; Goyal et al., 1987) and PCR technique and found negative; however it was positive by PCR for Rotavirus type B and C (Kapikian et al., 2001). The material was negative for *Isospora*, *Eimeria* and parasite ova by fecal flotation techniques.

The means for each parameter among specific treatment group with the respective *P* values derived from the statistical analyses are shown in Table 1. There was no statistical difference in *Cp* or coliforms quantity in dams' feces among treatment groups ($P > 0.05$) at farrowing. However group 1, which received the most intense feedback, had numerically greater shedding of both genera when compared to other study groups.

Colostrum analysis demonstrated no significant difference ($P > 0.05$) on either the total IgG (RID) or on the antibodies levels for alpha toxin (ELISA) among treatment groups. However, group 1 had numerically greater antibody levels when compared to other treatment groups. Interestingly, no antibodies for *C. difficile* toxin A (*TcdA*) or for toxin B (*TcdB*) were detected in colostrum from the study animals. There was no significant difference ($P > 0.05$) in the colostrum white cell count among groups. However, similar to the other variables, group 1 was found to have numerically greater results when compared to other groups.

Discussion

Although OCE has been widely utilized in swine operations for decades, there is a lack of scientific knowledge available validating benefit. This subject remains somewhat controversial among swine veterinarians because standardized protocols do not exist and questions rose about the efficacy or potential harmful effects.

In order to characterize and better understand the advantages and disadvantages of the OCE procedure, several variables were chosen to be evaluated in this pilot trial. These parameters are believed to be directly affected when animals are orally exposed when compared with animals that did not receive similar treatment. Feedback material characterization was crucial since the microorganisms present in the material are the main reason why this procedure is usually done. In this study, the feedback material was shown to contain a moderate growth of coliforms and a high to moderate growth of *Cp* which was expected since these organisms commonly habit the piglets' gastrointestinal tract and are commonly shedding in feces. The feedback material tested negative for Rotavirus type A in the ELISA test and PCR; however, it tested positive by a PCR for types B and C.

The main benefit expected from OCE is improving the immunological response of the dam which could enhance the passive immune protection offered to the piglets via colostrum and milk. White cells and IgG were assessed from colostrum samples in an attempt to verify the benefits. A white cell count and a total IgG quantification were performed on all colostrum samples but no significant difference was found between treatment groups. Other study have compared vaccinated and non-vaccinated sows placed in the same unit regarding to IgG levels in colostrum and they have concluded that the vaccination status did not influence total colostrum IgG (Arey et al., 2000). However, specific antibody titers are greater in vaccinated animals when compared to non-vaccinated (Le Dividich et al., 2005). To determine whether the difference among groups may be dependent on specific antibody amount rather than total antibody quantity, *Cp* alpha toxin ELISA and *Cp* alpha toxin and *C. difficle* A and B toxin neutralization were performed in order to quantify the specific antibodies. Alpha toxin titer was consistently high among all groups; therefore, it was not able to differentiate among treatment groups. The relatively high values among all groups could be explained by the fact that *Cp* is widely spread in the environment including soil, manure and water, as well as in the intestinal tract of humans and warm blood animals (Odendaal, 1994). An interestingly numeric trend was found in the toxin neutralization assay and in the ELISA; it shows the greater titers belonging to the groups which received the oral exposure when compared to the control group, as shown in figure 1 and figure 2.

Another observation to be made is the fact that exposure time might play a role in the immune response detected in colostrum. This trend could have been explained by the fact that the response to this toxin is dose-dependent and that perhaps oral exposure two weeks prior to parturition is not sufficient maximize the production and later transfer of the anti-toxin via colostrum. The impact of timing of exposure could play a very important role on the concentration of antibody in colostrum perhaps the exposure should occur weeks sooner. Since statistical evidence was not detected, those assumptions are simply speculations which should be investigated further. Interestingly, no titers of either *TcdA* or *TcdB* were detected from the samples.

Further studies should consider targeting specific antibodies for different diseases such as antibodies for coronavirus, rotavirus and some *E. coli* strains. Perhaps antibody measures for different endemic microorganism will have different results. Each farm may have different strains, disease pressure, ecology therefore extrapolation across farms should be made with care. Another line of investigation for further studies might evaluate the effect of OCE on the total amount of IgA throughout the lactation period. It has been well established that IgA is the dominant immunoglobulin throughout the lactation (Le Dividich, 2005). Another point is that the feedback presumably containing potential pathogens which will stimulate the mucosal immunity and therefore the response constitute of IgA is expected.

The results showed no statistical difference between the shedding of *Cp* and coliforms in feces of dams with the treatment. Many other microorganisms could be affected by the procedure, such as *C. difficile* or *Salmonella*, were not evaluated in this particular study. This is a hypothesis generating study in which the sample size was calculated and somewhat limited based on external factors and on the lack of previous data to support part of the experimental design calculation. A larger sample size or the choice of a farm facing current scours issues could potentially impact the measures. The variety of available diagnostic tests somehow limited the parameters evaluated however new tests are always been developed which open a new parameters that could be further evaluated. This study generated valuable information which will likely be used for further investigations and more elaborate experimental designs. Potential weaknesses and limitations of this study are important to

consider when interpreting the results. The diagnostic tests used to evaluate antibody levels in this study were developed for serum and therefore had to be modified for use with colostrum. These modifications have not been validated and as such, may have affected accuracy and interpretation. Another issue that was encountered was the lack of clinical disease during the experimental period therefore no clinical effects were measurable.

The objective of this paper was not to encourage or discourage the use of OCE, but instead, to identify parameters that could be used to evaluate this technique. The results were not able to identify significant differences in the measures used to assess the value of feedback. This study did not find any evidence of detrimental effects attributable to the procedure when evaluating *Cp* and other coliforms. The decision to implement or continue the use OCE will depend on the diseases present and farm-specific factors. Some of the pertinent factors to be considered in the decision process include an accurate diagnosis of the specific infectious diseases present in the farm thorough laboratory diagnostic investigation as well as the presence of compatible clinical presentation. If the diseases detected are considered to be endemic, are potential enteric pathogens, and associated with clinical disease then it is important to identify and prioritize those risk factors that may exacerbate disease expression. Risk factors include but are not limited to herd disease history, internal and external biosecurity, parity structure, the neonatal microenvironment, colostrum management, cross-fostering practices, the nutritional status and body condition of dams, the vaccination practices, and overall hygiene. The ability to consistently execute the OCE procedure should also be taken into consideration. Likewise, the collection of appropriate feedback material, the proper storage of the inoculums if necessary and the ability to follow the protocol have important roles in the success or failure of this technique. Although this study did not identify feedback value when attempting to control clostridial associated neonatal diarrhea, it is the first scientific investigation assessing the role of OCE as a method to enhance colostrum antibodies directed at C*ptA*. This information may be helpful to those veterinary practitioners who are attempting to manage *Cp* associated diarrhea and who are challenged with designing and implementing OCE strategies

Implication

1. In this study OCE had no affect on the total IgG amount in the colostrum from gilts.
2. No evidence for increased coliform shedding or *Cp* when OCE was performed on pregnant gilts. Neither does feedback timing appear to have an effect on the *Cp* and coliform levels of shedding.
3. Numerical trends suggest that OCE might influence the levels of specific antibodies such as anti-alpha toxin in colostrum. Similar feedback timing may play a role on the colostrum anti-toxin antibodies quantity.

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Tables and Figures

Table 1. Summary of Treatment Groups and timing of oral controlled exposure

Treatments	5 weeks*	4 weeks	3 weeks	2 weeks
Group 1	X†	X	X	
Group 2	X			
Group 3				X
Group 4				

Each treatment group was comprised of 10 gilts

* Weeks prior to farrowing,

† Oral controlled exposure applied

Table 2. Means of parameters among study groups with respective P values

Parameters	Group Means				
	1	2	3	4	*P* value
IgG	714	761	618	633	0.45
Cell count	326,667	252,000	246,667	314,000	0.78
Anti-alpha toxin	91.8	90.8	88.4	87.8	0.49
C. perfringens	2.1	1.7	1.9	2.0	0.73
Coliforms	2.8	2.6	2.7	2.6	0.67

IgG – Immunoglobulin G; Cell Count- white cell count in colostrum; Anti- alpha toxin-ELISA; *C. perfringens* and coliforms - shedding via feces at farrowing

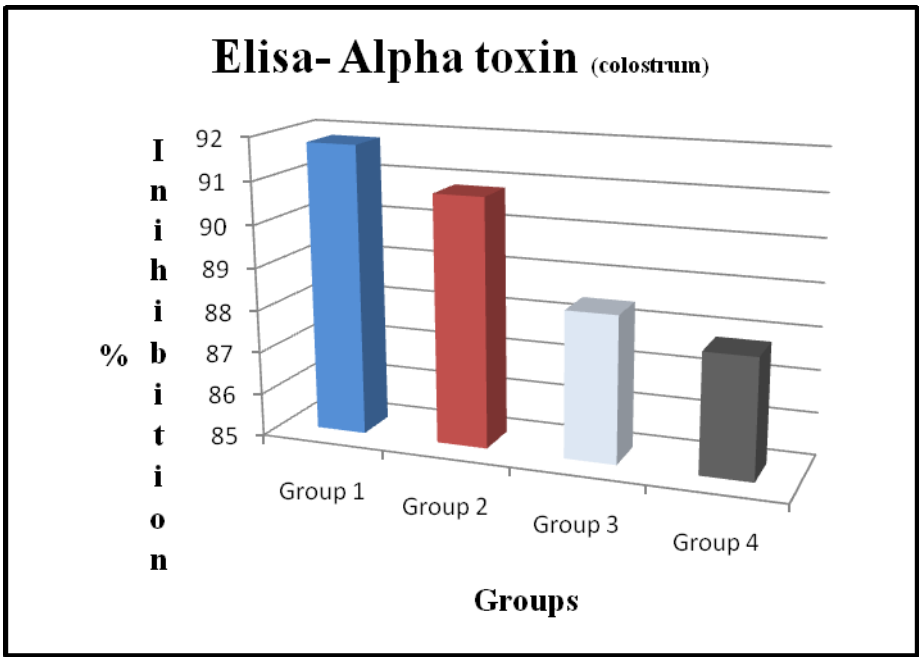

Figure 1. Clostridium perfringens anti alpha-toxin antibody measured by ELISA test

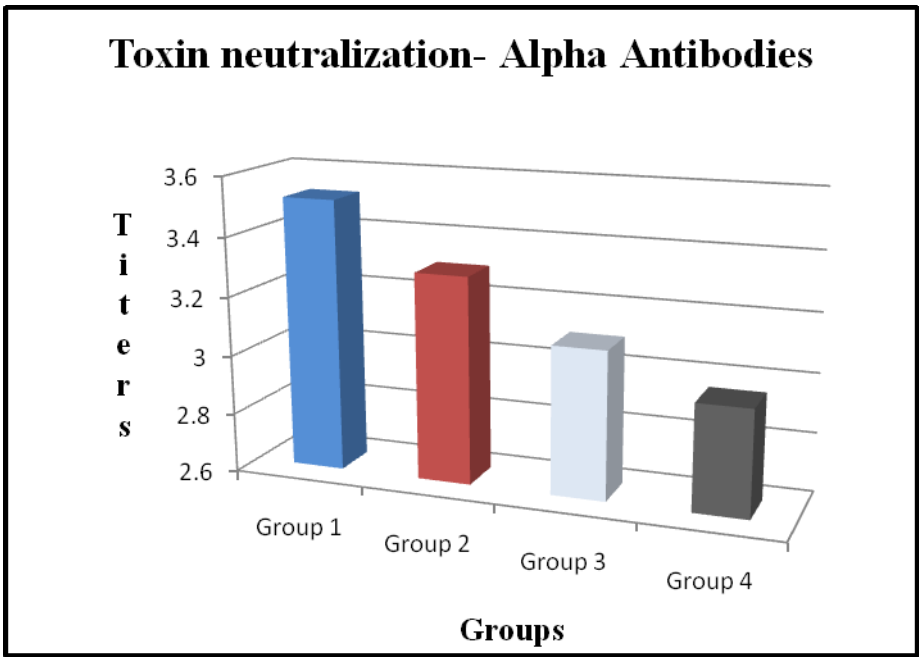

Figure 2. Clostridium perfringens anti alpha toxin antibody measured by toxin neutralization test

CHAPTER 4. *CLOSTRIDIUM PERFRINGENS* TRANSMISSION AND POSSIBLE STRATEGIES OF CONTROL

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Review

Clostridium perfringens was first described in 1891 by Alchame as *the Bacillus of Acute Rheumatism*. Since then, it has been known by different names such as *Bacillus phlegmoni, Bacillus cadaveris butyricus, Bacillus aerogenes capsulatus* etc *(Odendaal, 1994).* *Clostridium perfringens* (*Cp*) is gram positive, endospore forming and strictly anaerobic bacteria. *Clostridium perfringens* is divided into five types (A, B, C, D, E) based on the ability to produce one or more toxins. The four major toxins: alpha, beta, epsilon and iota (McDonel, 1986). The bacteria's virulence is associated with its ability to produce toxins and the specific characteristics associated with each particular toxin produced (Sayeed et al., 2008). The remainder of this paper will focus on *Cp* type A (*CptA*) and type C (*CptC*), since they are the main enteric clostridial pathogens of swine.

The major *CptA* toxins are the alpha toxin (CPA) and a recently described beta 2 (Bueschel et al., 2003; Waters et al., 2003). Recent studies have shown a correlation between pigs with clinical diarrhea signs and the Clostridium type A and beta 2 toxin production. Studies have shown over 90% of the *CptA* isolates recovered from neonatal swine with diarrhea are positive for beta 2 toxin in contrast to 11.1 % of age- matched controls without diarrhea (Bueschel et al., 2003). Beta 2 toxigenic strains have inconsistently reproduced disease in experimental infections which suggests more research is necessary in order to better understand the role of beta 2 in the disease pathology.

Clostridium perfringens type C produces two major toxins: CPA and beta toxin (CPB), the latter is a very lethal pore-forming toxin (Tweten, 2001). The clinical signs associated with *CptA* are mainly enteric including nonhemorrhagic mucoid diarrhea. Affected piglets develop

a pasty diarrhea, and frequently it is possible to observe a rough hair coat and fecal staining of the perineum (Johannsen et al., 1993a). The disease is observed within 48 hours of birth (Johannsen et al., 1993a) and clinical signs have been reproduced experimentally in gnobiotic colostrum-deprived pigs as well as conventional piglets (Johannsen et al., 1993a). The diarrhea can last up to five days and the majority of the piglets recover; however, in some animals the stunting consequences can be noticed throughout the nursing and grow-finish period (Songer and Taylor, 2006).

The disease associated with *CptC* has a completely different presentation; the most remarkable characteristic is the hemorrhagic diarrhea which is observed in acutely affected piglets. The clinical signs are commonly seen in the first three days of life; however it may appear in as little as12 hours after birth (Bergeland et al., 1966; Meszaros and Pesti, 1965). In *CptC* outbreak, the mortality rate can vary according to herd immunity status; however, there have been reported situations where100% individual litter and 50% herd mortality rates have been documented. (Bergeland et al., 1966; Hogh, 1967). The majority of acutely affected piglets die within one or two days due to profuse hemorrhagic diarrhea, severe intestinal necrosis, and rapid body condition loss. The severe disease condition will greatly impact the survivability since the piglets' ability to nurse, maintain body temperature and movement will be severely impaired. Gross lesions are remarkable in severity, with intestinal necrosis, hemorrhage, and emphysema a common finding in small intestinal segments. Microscopic examination reveals necrotic and nonfunctional villi in affected piglets. Disease associated with *CptC* can be presented as subacute and chronic cases. In these cases, piglets normally present a nonhemorrhagic diarrhea and remain active. However, depending on the disease severity, they will become thin and dehydrate over time; this will consequently affect the litter, weaning weight and mortality rate. The different disease presentations can be explained by herd immunity, piglet age (Niilo, 1988), and toxin levels produced (Niilo, 1988).

The newborn piglet is born with a sterile gastrointestinal tract but colonization by mixed populations of bacteria occurs within hours of birth (Ducluzeau, 1983; Mackie, I et al., 1999). Colonizing microbes are mechanically acquired by the piglets via oral contact within the dam's vaginal canal and perineum, environmental exposure, fecal exposure, oral contact

(suckling) and skin contact (Mackie, I et al., 1999; Smith, 1965). Lactobacilli, *Streptococcus*, *Fusobacterium*, *Cp* and *E. coli* are among the first organisms to colonize the intestine and colon (Pesti, 1962; Smith and Crabb, 1961; Wilbur et al., 1960). Smith (1961) demonstrated that *Cp* populations rapidly increase during the first day of life. In the first few hours after birth, he was able to recover 10^3 *Cp*/g of feces, and in the following hours saw a dramatic increase, with levels reaching up to 10^8-10^9 *Cp*/g (Smith and Crabb, 1961). The number of organisms/g constantly decreases as the pig gets older, reaching much lower levels of 10^2-10^3 *Cp*/g by the time pigs reached 23 weeks old (Smith, 1965; Smith and Crabb, 1961).

Several factors play a role in the dynamic succession of different microorganisms that make up the microflora. In addition to the early colonizing organisms mentioned, many other microbes compete for presence in microscopic niches in a succession process that will eventually establish the flora consisting of well over 500 distinct species of bacteria in the mature gastrointestinal tract (Artis, 2008). This process is very complex and can be affected by the dam's flora, the flora and bacterial dose in the environment, antimicrobial usage, ingesta qualities and quantities, and is modulated by acquired immunity from the dam and then by piglets' active immune responses (Ducluzeau, 1983; Mackie, I et al., 1999). The heavy *Cp* and *E. coli* growth in the first days of life might be explained by the fact that young piglets have a poor gastric acid production in the stomach where the pH varies between 5.3-5.9 (Smith, 1965). There is also a rapid generation time of those bacteria in the nutrient dense intestinal environment from a suckling piglet devoid of established flora. *Clostridium spp* are normally shedding in sow's feces. The generation time of *Cp* in particular is incredibly fast, evident by the ability to double the population in about eight minutes when in a optimal environment (Odendaal, 1994). Another study demonstrated that *CptC* can multiply to numbers approximately to 10^8 - 10^9 per gram of feces in only a few hours (Ohnuna et al., 1992). The main infection sources are considered to be piglet-to-piglet and shedding via dam's feces (Songer, 1996; Songer and Taylor, 2006) or spores already present in the environment. The persistent organism presence and its associated endospores in the environment are probably just as important as direct pig contact or dam fecal shedding.

Clostridium perfringens type A is consistently recovered both from the pigs' intestinal tract

and from its environment. The other types, such as B, C, D and E, are less likely to be recovered in the intestinal tracts from normal animals (Carter and Chengappa, 1991) but can eventually be found in the environment in areas where the disease is enzootic (Niilo, 1980). *Clostridium perfringens* type C is not commonly detected as part of the normal swine intestinal flora, but detection sensitivity and screening isolates costs which may underestimate true prevalence. In contrast, *CptA* is found in pigs' normal intestinal flora (Mansson and Smith, 1962) and can be routinely demonstrated in normal feces from pigs across all age classes.

The *CptA* pathogenesis in suckling piglet diarrhea is unclear at present. In affected piglets, the microorganism population can reach 10^8 - 10^9 in the ileum and jejunum Johannsen (1993) but throughout the studies Johannsen was not able to observe intestinal wall attachment or invasion (Johannsen et al., 1993b). The CPA and CPB2 production are believed to be associated with the pathogenesis; however there is a lack of information on the role they play. There might be a participation of others toxins which have not been yet described. No consistent lesions were found when purified CPA was inoculated in six hour old-piglets; however some piglets develop a mild villi edema (Johannsen et al., 1993a). Conversely, the pathogenesis associated with *CptC* is clear and early studies were able to demonstrate *Cpt*C attachment to jejunal epithelial cells at villous apices (Arbuckle, 1972; Walker et al., 1980b). The cellular desquamation and proliferation was also observed along the base membrane. Eventually, few microorganisms will penetrate the intestines causing mucosal emphysema in the tunica muscularis. The lesions are a result of the CPB necrotizing effect (Hogh, 1967; Warrack, 1963). This toxin is a protein that forms pores in the membrane of susceptible cells, which leads to swelling and cell lysis (Nagahama, 2003; Sakurai, 1978). Necrosis can include crypts, muscularis mucosa and submucosa. The bacteria can remain adhered to the necrotic cells and can be shed into the intestinal tract (Kubo and Watase, 1985).

There are two neonatal pig physiological characteristics which may account for the greater susceptibility to beta toxin. The first is physiological immaturity for production of trypsin and the second is the natural presence of protease inhibitors in colostrum. The immunoglobulins present in colostrum are sensitive to different proteolytic enzymes,

including trypsin (Stone et al., 1979). Therefore, the physiological aspects such as low trypsin levels in the stomach and intestines as well as the presence of trypsin-inhibitor in colostrum are important to prevent immunoglobulins proteolysis and absorption. Hence, the colostral immunoglobulin remains intact and can be transported from the intestinal lumen via the absorptive epithelial cells to the blood (Moog, 1979; Murata, 1977). However, this clever mechanism to prevent immunoglobulin digestion may be a risk factor for *Cp* associated disease. *Clostridium perfringens* beta toxin is a 35kDa protein, therefore would be expected to be normally degraded by endogenous trypsin levels which do not occur since trypsin is not substantially present in the neonatal small intestine (Sayeed et al., 2008).

Various methods are attempted to prevent and/or control these diseases. Harnessing the animals' immunological response appears to be a favored biological approach. Immuno-stimulation has been highly successful for *CptC* control. Commercial vaccines contain beta toxin toxoid as the target antigen and are given to the dam by injection at breeding or during gestation at least two to three weeks prior to farrowing (Kennedy et al., 1977b). The use of the vaccine usually eliminates the occurrence of the disease in the dam's offspring. There is only one commercial toxoid available in North America to prevent the disease caused by *CptA*. In a study conducted by (Hammer et al., 2008), sows and gilts were immunized with alpha toxoid associated with a proprietary dual adjuvant formulation and then given a booster vaccination 21 days later. Blood samples from vaccinated gilts were obtained 15 days after the second vaccination and their piglets were sampled at two and four days of age in order to assess the anti-alpha toxin antibody titers. The study results demonstrated that vaccinated gilts and their subsequent litters had significantly greater antitoxin titers than control animals. (Hammer et al., 2008). The clinical significance of this finding was not evaluated in this study due to the lack of a natural challenge or challenge model in this study. Even though the alpha toxoid will boost the anti-alpha toxin titer in vaccinated animals, the alpha toxin pathogenic role or other toxins that may be elaborated by *CptA* is not clear.

The expectation for vaccination of the dam is that it will boost the dam's immunity as well as improve the passive transfer of immunity to offspring. It follows that the outcome of any immunizing procedure is highly dependent on the colostrum intake by piglets. In swine, the

placenta does not allow for *in-utero* the transfer of antibodies; pigs have an epitheliochorial placenta which is impermeable to immunoglobulins, therefore, piglets are hypogammaglobulinemic or agammaglobulinemic at birth (Kim, 1975). Although the neonate piglets are able to produce an immune response to some antigens encountered by parenteral (Binns, 1967) and enteric routes (Redman et al., 1978), their gastrointestinal tract is sterile while in the uterus. In piglets, the gut closure occurs about 24 to 36 hours after birth which means that large macromolecule absorption ceases to occur (Lecce, 1973). Furthermore, the colostrum immunoglobulin concentration dramatically declines within the first few hours after farrowing (Bourne, 1969; Klobasa and Butler, 1987). Therefore, the timing, quantity, dose, and specific immune properties of the colostrum ingested by piglets plays an important role in the intervention procedures' outcome and ultimately in the piglets' health (De Passille´ and Rushen, 1989; Tyler et al., 1990). Alternative attempts to stimulate dam immunity include implementing oral control exposure (OCE) also known as feedback or by vaccination with either commercial or autogenous products.

Oral controlled exposure (OCE) is defined as the use of farrowing house-derived materials (usually feces) fed to pregnant animals' weeks before farrowing in order to increase exposure thereby boosting dams' immunity pre-farrowing as well as improving the efficacy of passive transfer of immunity. However, the credible data from properly controlled experiments on the efficacy of these procedures specific for *CptA* enteritis are scarce. Another appealing approach to control the disease may be to decrease the level of piglet's exposure. *Clostridium perfringens* type A is commonly found in sows' intestines at relatively low numbers but is constantly shed via feces to the environment. Cleaning and disinfecting of farrowing rooms is routinely performed in modern swine operations. These two procedures are very important, especially in farms where all in-all out age segregation management is adopted. Cleaning and disinfection performed between groups of pigs in order to prevent the exposure to microorganisms shed by the previous groups (Fotheringham, 1995; Owen, 1995; Tamasi, 1995). The cleaning process is important since it removes the organic material. This process will decrease the environmental bacterial and viral load as well as help the disinfection process. It is known that the organic material interferes with the efficacy of disinfectants by

either inactivating or decreasing the contact surface. In an attempt to enhance the cleaning practice, many farms have adopted hot water and detergent. The use of an appropriate disinfectant is very important since there is no ideal product for all situations. The selection should be based on disinfectant class, properties, label, field efficacy and cost (Amass, 2004). Scientific knowledge of the microorganism is essential since disinfectants are pathogen specific. *Clostridium perfringens* forms ovoid to eccentric spores and, although not an efficient sporulator, these spores are resistant to heat, some disinfectants and UV light (Songer and Taylor, 2006). Therefore the disinfectant selection is an important step, as such disinfectants have demonstrated efficacy against the bacteria itself and spores. There is considerable research published in human medicine literature regarding *C. difficile* disinfection. The conclusion supports the recommendation to use dilute solutions of hypochlorite (1,600 ppm available chlorine) for either routine environmental disinfection to reduce the incidence of *C. difficile* diarrhea (Wilcox, 2003), or in rooms with high *C. difficile* rates (Mayfield et al., 2000). Other options are the use of acidified bleach and regular bleach (5000 ppm chlorine) since they are capable of inactivating 10^6 *C. difficile* spores in $\leq$ 10 minutes (Wilcox and Fawley, 2000). Lastly, it has been demonstrated that 2% glutaraldehyde (Dyas and Das, 2003; Hughes et al., 1986; Rutula et al., 1993; Wullt et al., 2003) and peracetic acid (Block, 2004; Dyas and Das, 2003) reliably kill *C. difficile* spores using exposure times of 5–20 minutes.

The goal of cleaning and disinfecting is not to achieve sterility, as theoretically no current protocols used on swine operations are able to eliminate all of the bacteria in a farm environment. Yet hygiene cannot be overestimated in the farrowing environment. Most of the time the value of these procedures is underestimated by the farm personnel, therefore an education program which emphasizes the benefits as well as a training are highly encouraged. Ideally, the cleaning process should remove all organic material from the farrowing crates. High pressure water use throughout power washing facilitates the organic material remove and is a widely used process on swine operations. The disinfectant of choice should be based on the resistant characteristics of the microorganism, including spores. Product such as Virkon®S, Trifectant® (Peroxygens), Parvosan® (Formaldehyde and

Quaternary Ammonium) and DC&R® (Formaldehyde and Quaternary Ammonium) are products labeled to control *Cp* (National Biosecurity Resource Center, 2010). Following disinfection, it is recommended that a drying period be implemented so the disinfectant has sufficient time to act. Hygiene throughout the suckling period is a good practice. Cleanliness of the crates' floor should be continuously kept by scraping feces from behind the sows as that will be the first surface piglets are exposed to after birth.

The use of antimicrobial and probiotics in feed of pre-farrowing animals are other alternatives to approach the piglet exposure. Antimicrobials in swine feed is widely implemented among producers for different reasons such as improvement of growth rate, disease control and prevention and health treatment issues. The administration of antimicrobials to sows pre-farrowing may benefit piglets in two ways: first, by reducing the *Cp* shedding at farrowing, and second, by the partial product transfer through milk (Sbiraki et al., 2003). However much attention has recently been devoted to this topic due to an increased public concern focused on the possible association with antimicrobial resistance. In response to this concern, studies in which sows pre-farrowing were supplemented with either prebiotics or probiotics have been conducted and results indicated a reduction of *Cp* shedding at farrowing (Krueger et al., 2002; Nagamine et al., 1998). Prebiotics can be defined as nondigestible food, primarily oligosaccharides. They are normally not digested or absorbed by the pig providing readily nutrients which will selectively favor the growth or certain species of bacteria in the gastrointestinal tract (Gibson et al., 1995; Zimmermann et al., 2001). Probiotic are live microorganisms dietary supplementation such as *Lactobacillus acidophilus*, *Enterococci faecium*, *Bacillus* species, *Bifidobacterium bifidum* with the objective of improving the intestinal microflora balance (Fuller, 1989; Simon et al., 2003). Figure 1 is a diagram which includes several factors and their association with immunity and exposure which are believed to influence disease occurrence. Some of these factors have been investigated and their relevance to the disease prevention is clearly understood; however, some factors have not been completely investigated and therefore, more research is necessary in order to understand the complexity of interactions and their specific importance in the prevention and control of the disease. Even though there is some research on these

topics; unequivocal, well-designed studies to support their efficacy remain lacking.

Conclusions

Disease associated with *CptC* can be easily diagnosed throughout the case history and with gross and microscopic lesions. The disease is usually prevented by vaccinating sows pre-farrowing with commercial toxoid. The immunization of sows and eventually piglet's protection is highly correlated with the quality and quantity of colostrum intake.

In contrast, several aspects of neonatal enteritis associated with *CptA* are still unclear at this point. The diagnosis is normally based on the isolation of high numbers of the microorganism in the small intestine and absence of other enteric pathogens. The lack of microscopic lesions and lack of pathogenesis understanding of the mechanisms for the disease makes the diagnosis and prevention efficacy very equivocal. Based on the available information, there is no best option (silver bullet) for prevention; however, there is a combination of measures that could impact the disease occurrence. Good management practices are desirable in order to provide the correct microenvironment for the piglets at birth which will maximize the piglets' colostrum ingestion as well as reduce the levels of exposure early in life.

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Tables and Figures

Table 1. Clostridium perfringens major toxins

Major toxins				
Type	Alpha	Beta	Epsilon	Iota
A	X			
B	X	X	X	
C	X	X		
D	X		X	
E	X			X

Table 1. *Clostridium perfringens* toxinotypes

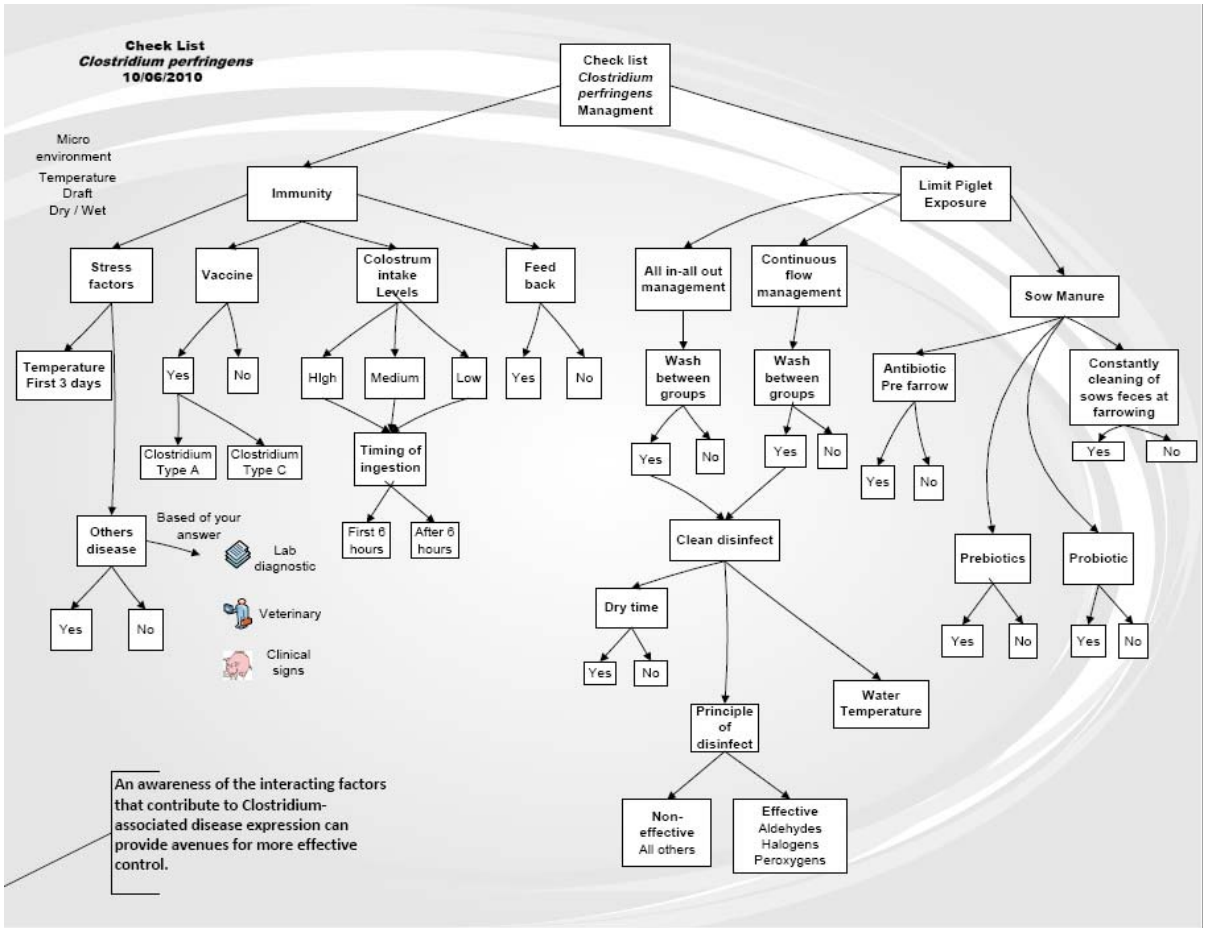

Figure 1. Chart including important factors believed to impact the control of clostridial associated disease in swine operations.

GENERAL CONCLUSIONS

The U.S swine industry and medicine has developed incredibly over the years with the introduction of new technologies and scientific knowledge. However, this advancement is very limited to specific topics thereby neglecting some important areas such as enteric diseases associated with neonate piglets. The objective of this thesis was to focus on the enteric disease associated with neonate piglets with a special focus on *Clostridium perfringens* (*Cp*) and the technique of oral control exposure (OCE).

The oral control exposure trial result interpretation showed in this particular study OCE had no affect on the total amount of IgG in the colostrum of gilts. Furthermore, there was no evidence that the use of OCE in gilts increased the shedding of coliforms or *Cp*. The results also showed that timing of feedback does not appear to have an effect on the levels of shedding of *Cp* and coliforms. Numerical trends suggest that OCE might influence the levels of specific antibodies such as anti-alpha toxin in colostrum. Similarly, the timing of feedback may be a factor in the quantity of anti-toxin antibodies in colostrum.

Further studies should target specific antibodies for different diseases such as antibodies for coronavirus, rotavirus and some strains of *E. coli*. Perhaps the measure of antibodies for different endemic microorganisms will have different results. Each farm may have different strains, disease pressure, ecology therefore extrapolation across farms should be made with care.

Another line of investigation to explore is the effect of OCE on the total amount of IgA throughout the lactation period. It has been well established that IgA is the dominant immunoglobulin throughout the lactation. An additional point is that the feedback presumably containing potential pathogens could possibly stimulate the mucosal immunity and therefore generate a response largely made up of IgA.

The main idea is that because of the way society is evolving, it is going to be harder to justify a procedure such as oral control exposure where animal feces are fed back to other animals which in the end will be used as a human food source. Veterinarians and Animals Scientist have the obligation to scientifically investigate the advantages and possible consequences of this practice in order to explain to society what, how and why we perform such techniques.

The *Cp* review provided valuable information which will likely support veterinarians in the field in the understanding and in intervention development to possibly prevent or control this disease. The conclusion from the *Cp* transmission review clearly outlines the necessity of more resources and investment in this topic. The apparent number of questions to be answered as well as the lack of scientific knowledge in determined areas is easily noticeable. Redirecting resources and increasing awareness about the potential impact that neonate enteric disease could have on the industry are believed to be the best ways to approach the issue.

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